American Journal of Medical Genetics 64:404–407 (1996)

Segregation of the Fragile X Mutation From a Male With a Full Mutation: Unusual Somatic Instability in the FMR-1 Locus

Marios Kambouris, Karen Snow, Stephen Thibodeau, Denise Bluhm, Michael Green, and Gerald L. Feldman

Henry Ford Hospital, Detroit, Michigan (M.K., D.B., G.L.F.); Mayo Clinic, Rochester, Minnesota (K.S., S.T., M.G.)

Fragile X syndrome is associated with an unstable CGG-repeat in the FMR-1 gene. There are few reports of affected males transmitting the FMR-1 gene to offspring. We report on a family in which the propositus and his twin sister each had a full mutation with abnormal methylation. Their mother had an FMR-1 allele in the normal range and a large premutation, with normal methylation. The maternal grandmother had two normal FMR-1 alleles. The maternal grandfather had an unusual somatic FMR-1 pattern, with allele size ranging from premutation to full mutation. No allele was detectable by PCR analysis. Multiple Southern blot analyses identified a hybridization pattern that originated at a distinct premutation band and extended into the full mutation range. Methylation studies revealed a mosaic pattern with both unmethylated premutations and methylated full mutations. This individual declined formal evaluation but did not finish high school and has difficulty in reading and writing. The size of the premutation FMR-1 allele passed to his daughter is larger than his most prominent premutation allele. This is most likely due to gonadal mosaicism similar to that in his peripheral lymphocytes. Alternatively, this expansion event may have occurred during his daughter's early embryonic development and this large premutation allele is mitotically unstable. This pattern of FMR-1 alleles in a presumably mildly affected male is highly unusual. These findings are consistent with the absence of transmission of a full fragile X mutation through an expressing male. Studies of tissue specific FMR-1 allele expansion and FMR-1 protein expression on this individual should help to determine the correlation of the molecular findings with the phenotypic effects.

KEY WORDS: fragile X syndrome, FMR-1 locus, somatic mosaicism

INTRODUCTION

Fragile X syndrome is the most common inherited cause of mental retardation in males, with an estimated frequency of one in 1,000 individuals [Verkerk et al., 1991; Kremer et al., 1991]. It is caused by amplification of a (CGG)n trinucleotide repeat in the FMR-1 gene at Xq27.3, and is associated with hypermethylation of a CpG island that precedes the open reading frame of the gene [Rousseau et al., 1991; Heitz et al., 1991, 1992; Vincent et al., 1991]. The normal number of (CGG)n repeats is polymorphic and varies from 6 to approximately 50 [Fu et al., 1991]. In fragile X syndrome there is progressive amplification of the (CGG)n repeat from unstable premutations (50–200) to the full mutation (>200 repeats). Expansion of the premutation to the full mutation is strictly maternal [Heitz et al., 1992; Yu et al., 1992]. Penetrant fragile X males show repression of FMR-1 transcription, and the absence of FMR-1 protein is thought to contribute to the fragile X phenotype [Verheij et al., 1993].

Although most males with the fragile X syndrome do not reproduce, eight clinically affected fragile X males are reported who had offspring [Laird, 1991; Mulley et al., 1992; Willems et al., 1992; Rousseau et al., 1994]. Molecular studies of the FMR-1 locus are available in only three of these affected males [Mulley et al., 1992; Willems et al., 1992; Rousseau et al., 1994]. While these patients had FMR-1 alleles of various sizes and patterns, each of their daughters (total of three) inherited

Received for publication May 31, 1995; revision received December 1, 1995.

Karen Snow is now at Molecular Genetics Laboratory, Auckland Hospital, Private Bag 92024, Auckland, New Zealand.

Address reprint requests to Dr. Gerald L. Feldman, Medical Genetics and Birth Defects Center, Henry Ford Hospital, 2799 West Grand Boulevard CFP-4, Detroit, MI 48202-2689.

premutation sized alleles only and were clinically normal. Of 11 obligate carrier daughters (including the 3 in whom molecular studies were done), intelligence was reported to be normal in 8 and probably subnormal in the remaining 3, but without evident mental retardation. None of these 11 females expressed the fragile X syndrome at the clinical or cytogenetic levels. Reyniers et al. [1993] showed that the full fragile X mutation was absent in the sperm cells of four fragile X patients and only premutation size alleles were present. They suggested that sperm from fragile X affected males contain only premutation size alleles. None of those four patients had reproduced.

In this report we present a family that was ascertained through the propositus, who has fragile X syndrome. His twin sister (also diagnosed with fragile X syndrome), their parents, and maternal grandparents were studied to determine the size and transmission pattern of their FMR-1 alleles. The propositus and his sister each had a full mutation. Their mother had a very large premutation with normal methylation. The maternal grandmother had two normal FMR-1 sized alleles while the maternal grandfather had a continuous distribution of FMR-1 alleles ranging from a distinct premutation to full mutation. These studies indicate that a premutation allele was passed to his unaffected daughter, which then expanded to a full mutation in her offspring.

MATERIAL AND METHODS

Case Presentation

The maternal grandmother reportedly has normal physical and intellectual development. The maternal grandfather was not examined. According to his daughter, he has large ears and a long face. He did not finish high school, has difficulty in reading and writing, but has lived a stable and productive life. He has a strong maternal family history of males with mental retardation, learning disabilities, or attention deficit disorders. His daughter has normal intelligence, joint laxity, and mitral valve prolapse. The propositus has developmental and speech delay and hyperactivity. He is currently in a pre-kindergarten classroom. He has typical fragile X findings, including a prominent forehead, long and narrow face, square chin and high arched palate. His twin sister also has developmental delay, although milder than her brother, speech delay, and echolalia. She has a prominent forehead, long and narrow face, square chin, and high arched palate. She is in a regular kindergarten class.

Molecular Studies

High molecular weight genomic DNAs were prepared from whole blood with the non-organic DNA extraction kit (Oncor Inc., Gaithersburg, MD) according to the protocol provided by the manufacturer. Southern blot analyses were performed according to standard procedures [Sambrook et al., 1989]. All Southern blot gels were 1% agarose. Southern blot analyses were performed by genomic DNA digestion with various restriction enzymes and hybridization with either the pE5.1 probe [Fu et al., 1991] or the StB12.3 probe [Snow et al., 1992, 1993].

Two hundred nanograms of genomic DNA were amplified using the polymerase chain reaction (PCR) with primers FMR-A and FMR-B [Innis et al., 1991]. PCR products were separated on a sequencing 7 M Urea/6% Polyacrylamide gel and detected by autoradiography. The number of CGG repeats for each FMR-1 allele was estimated by comparing the size of the amplified DNA fragments to the size of sequenced single-stranded M13mp18 DNA.

RESULTS

The maternal grandmother has a hybridization band representing normal sized FMR-1 alleles at ~1.0 kb for Pst I/pE5.1, and ~5.2 kb for Hind III/StB12.3. PCR analyses shows that she has FMR-1 alleles with 32 and 33 CGG repeats. The maternal grandfather has a mosaic pattern of hybridization ranging from unmethylated premutation to full mutation, with a smear beginning at ~100 CGG repeats (estimated from Southern blot analyses) for Pst I/pE5.1 (Fig. 1) and a distinct band for

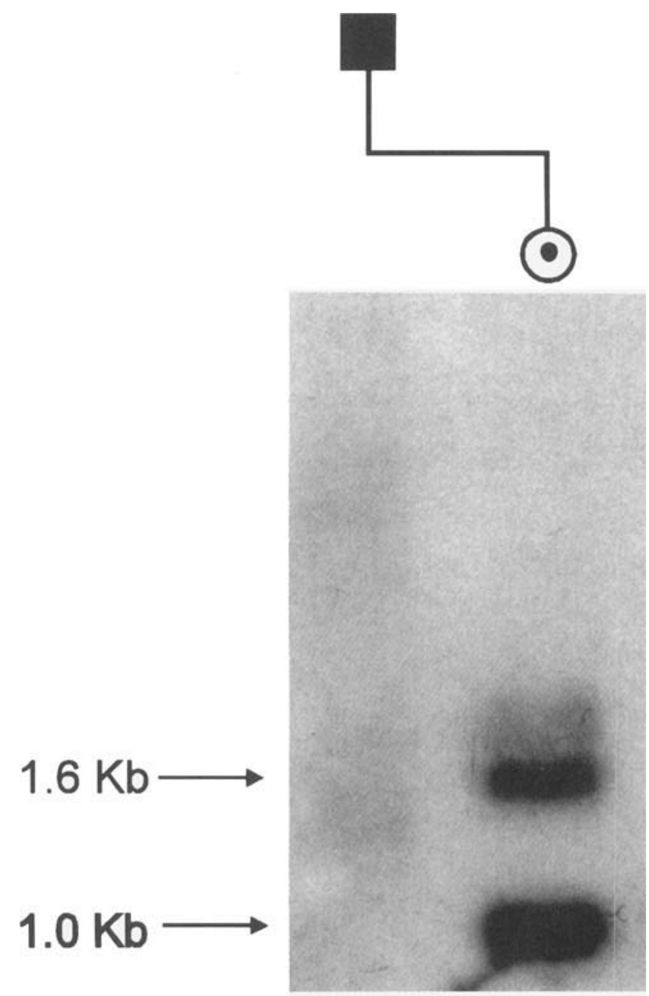

Fig. 1. Southern blot of Pst I digested genomic DNAs hybridized to the pE5.1 fragile X probe. The normal sized FMR-1 allele is ~1.0 kb. Expansions at ~1.3 kb and ~1.6 kb are indicated. The maternal grandfather is in the first lane; his daughter is in the second lane.

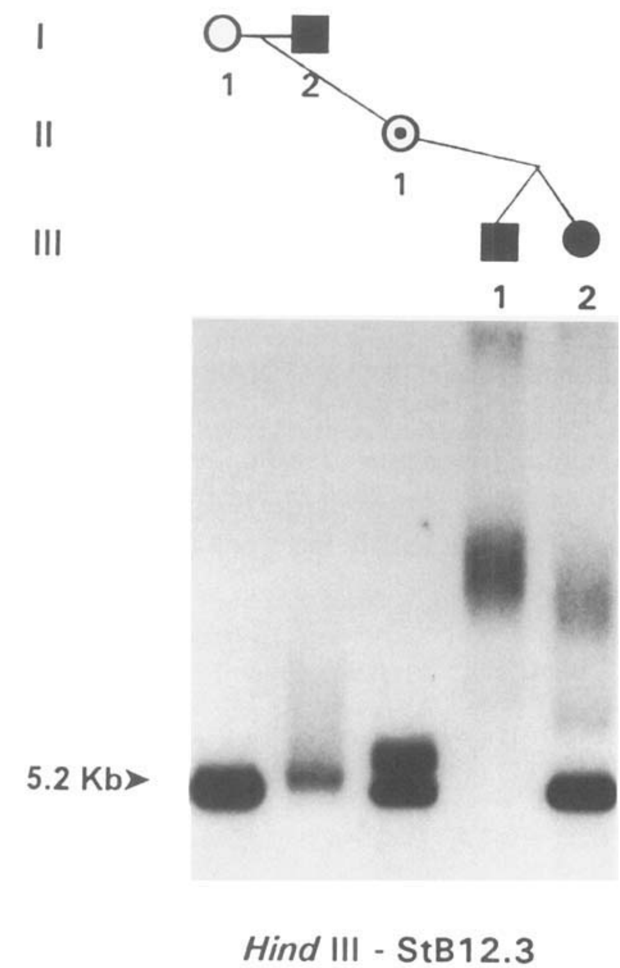

Fig. 2. Southern blot of Hind III digested genomic DNAs hybridized to the StB12.3 fragile X probe. The normal FMR-1 allele is ~5.2 kb. Individual I-2 shows expansion to premutations and full mutations; Individual II-1 shows a premutation expansion; Individuals III-1 and III-2 both show expansions to full mutations.

Hind III/StB12.3 (Fig. 2, I-2) and a null PCR allele. The propositus' mother has one normal FMR-1 allele with 33 CGG repeats, and a very large (unmethylated) premutation which was larger than her father's most prominent band. (Figs. 1, 2, II-1). The propositus has a full mutation (null PCR allele) (Fig. 2, III-1). His sister has one normal FMR-1 allele with 30 CGG repeats and a full mutation (null PCR allele) which appears to be slightly smaller than her brother's (Fig. 2, III-2). Each of their full mutations has greater than 1,300 CGG repeats. Methylation studies in the grandfather were difficult to interpret, but appeared to show a mosaic pattern with both unmethylated premutation alleles and methylated full mutation alleles (data not shown).

DISCUSSION

Molecular studies of the FMR-1 locus have been reported in only three affected males with a full fragile X mutation who have reproduced [Mulley et al., 1992; Willems et al., 1992; Rousseau et al., 1994]. In the report by Mulley et al. [1992], the affected patient had a full mutation in his peripheral lymphocytes while his two daughters had premutation-size alleles only and were clinically and cytogenetically normal. In the report by Willems et al. [1992], the patient had both clinical and cytogenetic evidence of fragile X syndrome. At the molecular level, he had a discontinuous pattern with a large full mutation smear and a premutation band. His daughter did not have any clinical or cytogenetic evidence of fragile X syndrome, and inherited her father's premutation FMR-1 allele, which subsequently expanded to a full mutation in her affected son. The molecular findings in that patient are not unusual, since approximately 15% of affected male fragile X patients have a mosaic pattern with a band in the premutation range and a smear in the full mutation range [Rousseau et al., 1991]. The patient presented by Rousseau et al. [1994] had cytogenetic evidence of fragile X syndrome and some phenotypic findings of the syndrome but no mental impairment. At the molecular level, he had a full mutation plus a wide range of premutations. His sperm cells had FMR-1 alleles of only one specific premutation size (at the lowest end of the premutations present in his lymphocytes), which was transmitted to his daughter. Both patterns are different than that seen in individual I-2, who has a continuous uninterrupted distribution of FMR-1 alleles spanning a range from pre- to full-mutation.

Our report describes a three generation family with an unstable FMR-1 allele that undergoes significant expansion from a carrier female to her affected offspring. The unusual hybridization pattern seen in the propositus' maternal grandfather probably reflects the presence of a premutation size allele (~100 CGG repeats) in most of his peripheral lymphocytes and a smaller number of cells with FMR-1 alleles that vary in size from premutation to full mutation. He has a strong maternal family history of males with various degrees of mental retardation. His learning difficulties are likely due to the presence of some cells with expanded FMR-1 in critical tissues such as the brain. The premutation size allele passed to his daughter is larger than his most prominent premutation. Since significant expansion of the FMR-1 locus has not been described in a male to female transmission, the size of the transmitted allele from this individual suggests the presence of germline mosaicism. Gonadal mosaicism would imply sperm cells containing FMR-1 alleles of various sizes, with a large premutation FMR-1 allele contained in the single sperm cell passed to his daughter. It is unknown if the gonadal mosaicism is confined to the premutation range or if it extends to include sperm cells with the full fragile X mutation. Our case suggests germline mosaicism for the apparent transition of a premutation from an affected male to his daughter, first proposed as a possibility by Willems et al. [1992].

Reyniers et al. [1993] suggested that the sperm of affected fragile X males do not carry the full fragile X mutation. In their study, gonadal tissues from four fragile X males were examined. In their peripheral lymphocytes, three had only full mutation FMR-1 alleles,

whereas the fourth had FMR-1 allele sizes in both the premutation and full mutation range. Sperm cells from this last patient contained only premutation FMR-1 alleles as did two of the other three patients examined. One patient, although he only had a full mutation allele in his peripheral lymphocytes, had both premutation and full mutation alleles in tissue obtained from a testicular biopsy. In our family, the molecular findings in individual I-2 suggest that at least some of his sperm cells contained large premutations, one of which was passed to his daughter. Another explanation is that individual I-2 transmitted a premutation allele similar to his smaller, most prominent band, which then expanded during his daughter's early embryonic development. This latter explanation is consistent with the hypothesis that expansion of the FMR-1 locus is a post-zygotic, early embryonic event that occurs after gamete differentiation in females. Similar expansions in CGG repeat number ranging from 10 to ~60 repeats have been seen in other families analyzed in our laboratories.

In summary, the FMR-1 alleles in fragile X males reported in the literature thus far either show a discontinuous distribution with distinct bands in the premutation range and full mutation smears or full mutation smears only. In contrast, our patient shows a continuous uninterrupted distribution of FMR-1 alleles that span the premutation and full mutation range. The exact mechanism and timing of FMR-1 amplification and the absence of the fragile X mutation in the sperm of affected males has not yet been resolved. Molecular studies that would identify the nature of the FMR-1 locus in different tissues (including gonadal tissues) as well as tissue specific expression studies for the FMR-1 protein, would be valuable in further contributing to the understanding of the timing and mechanism of the trinucleotide repeat expansion and correlation of the molecular findings with the phenotypic effects.

ACKNOWLEDGMENTS

The authors would like to thank the family for their cooperation and willingness to participate in this study and to Dr. Daniel Van Dyke for his review and useful comments on this manuscript.

REFERENCES

Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkerk AJ, Holden JJ, Fenwick RG, Warren ST, Oostra BA, Nelson DL, Caskey CT (1991): Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the Sherman paradox. Cell 67:1047–1058.

Heitz D, Rousseau F, Devys D, Saccone S, Abderrahim H, Le Paslier D, Cohen D, Vincent A, Toniolo D, Della Valle G, Johnson S, Schlessinger D, Oberle I, Mandel JL (1991): Isolation of sequences that span the fragile X and identification of a fragile X-related CpG island (published erratum appears in Science (1991) 252:494). Science 251:1236–1239.

Heitz D, Devys D, Imbert G, Kretz C, Mandel JL (1992): Inheritance of the fragile X syndrome: Size of the fragile X premutation is a major determinant of the transition to full mutation. J Med Genet 29:794–801.

Innis MA, Gelfand DH, Snissky JJ, White TJ (1990): "PCR Protocols: A Guide to Methods and Applications." San Diego: Academic Press, Inc.

Kremer EJ, Yu S, Pritchard M, Nagaraja R, Heitz D, Lynch M, Baker E, Hyland VJ, Little D, Wada M, Toniolo D, Vincent A, Rouseau F, Schlessinger D, Sutherland GR, Richards RI (1991): Isolation of a human DNA sequence which spans the fragile X. Am J Hum Genet 49:656–661.

Laird CD (1991): Possible erasure of the imprint of a Fragile-X chromosome when transmitted by a male. Am J Med Genet 38:391–395.

Mulley JC, Yu S, Gedeon AK, Donnelly A, Turner G, Loesch D, Chapman CJ, Gardner RJM, Richards RI, Sutherland GR (1992): Experience with direct molecular diagnosis of fragile X. J Med Genet 29:368–374.

Reyniers E, Vits L, De Boulle K, Van Roy B, Van Velzen D, de Graeff E, Verkerk AJ, Jorens HZJ, Darby JK, Oostra B, Willems PJ (1993): The full mutation in the FMR-1 gene of male fragile X patients is absent in their sperm. Nature Genet 4:143–146.

Rousseau F, Heitz D, Biancalana V, Blumenfeld S, Kretz C, Boue J, Tommerup N, Van der Hagen C, DeLozier-Blanchet C, Croquette M-F, Gilgenkrantz S, Jalbert P, Voelckel M-A, Oberle I, Mandel J-L (1991): Direct diagnosis by DNA analysis of the fragile X syndrome of mental retardation. N Engl J Med 325:1673–1681.

Rousseau F, Robb LJ, Rouillard P, Kaloustian VMD (1994): No mental retardation in a man with 40% abnormal methylation at the FMR-1 locus and transmission of sperm cell mutations as premutations. Hum Mol Genet 3:927–930.

Sambrook J, Fritsch EF, Maniatis T (1989): "Molecular Cloning: A Laboratory Manual," 2nd edition. Nolan C (ed). New York: Cold Spring Harbor Laboratory Press.

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster Sh, Thibodeau SN (1992): Analysis of mutations at the fragile X locus using the DNA probe Ox1.9. Am J Med Genet 43:244–254.

Snow K, Doud LK, Hagerman R, Pergolizzi RG, Erster Sh, Thibodeau SN (1993): Analysis of a CGG sequence at the FMR-1 locus in fragile X families and in the general population. Am J Hum Genet 53:1217–1228.

Verheij C, Bakker CE, de Graaff E, Keulemans J, Willemsen R, Verkerk A-J, Caljaard H, Reuser AJ, Hoogeveen AT, Oostra BA (1993): Characterization and localization of the fMR-1 gene product associated with fragile X syndrome. Nature 363:722–724.

Verkerk AJ, Pieretti M, Sutcliffe JS, Fu, Y-H, Kuhl DPA, Pizzuti A, Reiner O, Richards S, Victoria MF, Zhang F, Eussen BE, van Ommen G-JB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST (1991): Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65:905–914.

Vincent A, Heitz D, Petit C, Kretz C, Oberle I, Mandel J-L (1991): Abnormal pattern detected in fragile-X patients by pulsed-field gel electrophoresis. Nature 349:624–626.

Willems PJ, Van Roy B, De Boulle K, Vits L, Reyniers E, Beck O, Dumon JE, Verkerk A, Oostra B (1992): Segregation of the fragile X mutation from an affected male to his normal daughter. Hum Mol Genet 1:511–515.

Yu S, Mulley J, Loesch D, Turner G, Donnelly A, Gedeon A, Hillen D, Dremer E, Lynch M, Pritchard M, Sutherland GR, Richards RI (1992): Fragile-X syndrome: Unique genetics of the heritable unstable element. Am J Hum Genet 50:968–980.